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Fat Composition and *in vitro* Oxygen Consumption of Marrow from Fed and Fasted Rabbits

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Introduction

The polyunsaturated fatty acids are generally regarded as essential in the mammalian diet (1), but the role these acids play in maintaining the organism in a state of health and the processes by which they are ultimately consumed are almost completely unknown. Previous work has indicated that unsaturated fats are present in rabbit marrow and that they are of concern because of their capacity to undergo autoxidation, thereby potentially introducing an artifact into *in vitro* oxygen consumption measurements (2). The recent development of an exact spectrophotometric method of analysis of small quantities of fat (3) has made possible a comparison of oxygen consumption with fatty acid content and has led to the present description of marrow fat from fed and fasted rabbits.

Methods

For this investigation normal white, male, New Zealand rabbits were used. All animals were approximately 6 months old. Six control rabbits were allowed to feed at will on a diet composed of fresh vegetables and a commercial feed.[4] The fresh

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[4] The feed manufacturer was unable to supply the exact percentages of the various component meals, but guaranteed the following composition: crude protein

vegetables were given once or twice weekly, and the commercial feed which supplied the bulk of the diet contained ground oats, ground yellow corn, alfalfa meal, soybean meal, cottonseed meal, linseed oil meal, and vitamin and mineral supplements. Six experimental animals were placed on a no-food, unlimited water regimen for 4–11 days during which they lost an average of 28% of their original body weight. Bone marrows from the femora, tibiae, and humeri were pooled and gently pressed into a homogeneous mash. Weighed samples in Warburg vessels were suspended in isotonic Ringer phosphate at pH 7.4, gassed with 100% oxygen, and shaken at 38°C. Oxygen consumption was determined in the conventional manner. All manometric experiments were of 4 hr. duration unless specifically described as otherwise. A second weighed portion of marrow mash was analyzed for iron by the method of Kennedy (4). A third weighed sample was extracted with alcohol and ether and analyzed spectrophotometrically by the method of Herb and Riemenschneider (3, 5). In one sample, prolonged extraction in a Soxhlet apparatus indicated that the routine alcohol and ether extractions removed 99.2% of the marrow fat. From the iodine number (half-hour Wijs method) and the percentages of the polyunsaturated fatty acids in the extracted fat, the per cent of oleic acid in the sample was computed. Per cent of total unsaturated fatty acids are represented by the sum of the percentage values for all the unsaturated fatty acids. If one assumes that all the acids are in the form of triglycerides, approximately 95.7% of the total weight is attributable to the fatty acid components. The per cent of total saturated acids is therefore 95.7% minus the per cent of total unsaturated fatty acids.

In the next section the lipide content of marrow is expressed in two ways. All the figures describe marrow fat in terms of milligrams of fatty acid per gram of marrow. Table II describes the composition of marrow fat as the average per cent of each fatty acid component relative to the gross fat extracted from the marrows.

Results

Data on the composition of marrows from fed and fasted rabbits are shown in Table I. Column 5 shows the effect of fasting on the fat content of marrow. It will be seen that under conditions of good nutrition the marrow may be composed of as much as 38% by weight of fat, whereas in fasting the amount may decline to less than 1%. One fasted animal (rabbit 12), however, was exceptional in that its marrow contained more fat than did the marrow of one of the normally fed animals (rabbit 1). It is also evident from col. 4 that marrow weight does not decline on fasting in spite of marked fat depletion. Column 7 shows that the per cent of the fat-free, dry weight component is approximately the same in

(min.), 15.0%; crude fat (min.), 2.0%; crude fiber (max.), 18.0%; N.F.E. (min.), 46.0%. Our analyses showed the composition of the feed fat to be: linoleic acid, 45.3%; linolenic acid, 6.4%; arachidonic acid, 1.4%; pentaenoic acid, 0.3%; oleic acid, 16.7%; total unsaturated acids, 70.0%; total saturated acids, 25.7%; iodine number, 120.7.

TABLE I

Comparison of the Composition of Bone Marrows from Fed and Unfed Rabbits

Exptl. No.	Wt. of rabbit	Time fasted	Total wt. of marrow taken[a]	Total fat in marrow	Total poly-unsaturated fatty acids	Fat-free dry wt.
	kg.	*days*	*g.*	%	*g.*	%
			Fed rabbits			
1	1.68	0	4.14	9.3	0.12	13.3
2	3.07	0	7.09	11.3	0.36	15.9
3	1.60	0	2.49	20.5	0.15	—
4	—	0	3.82	24.2	0.39	—
5	—	0	4.08	32.6	0.53	—
6	—	0	3.38	38.6	0.56	—
			Fasted rabbits			
7	1.32	11	3.59	0.9	0.01	13.6
8	2.22	9	4.06	2.0	0.03	16.6
9	1.53	8	2.76	5.0	0.05	13.6
10	2.10	8	4.80	5.6	0.09	14.3
11	—	4	4.83	8.3	0.13	14.7
12	1.56	8	4.22	15.4	0.21	16.1

[a] From the femora, tibiae, and humeri.

fed and unfed rabbits. Unfortunately the data are not complete on the fed animals in this series, but in five other fed rabbits, data from which are not otherwise a part of this study, the per cent of fat-free dry weight materials were 18.5, 11.9, 10.0, 12.9, and 12.7%. It appears from this auxiliary information combined with that from the present work that the fat-free materials represent a relatively constant fraction of the total weight of the raw marrow. During fasting the space previously occupied by fat apparently becomes filled with fluid (6). Column 6 shows the total polyunsaturated fatty acids recovered from the marrow fat of each animal. There is a noteworthy depletion of these substances during fasting, details of which are shown in Figs. 1–4. In these figures the numbers above each set of vertical columns represent the average for that group.

Table II shows the change in average percentage distribution of the various fatty acids in the fat extracted from the marrows of fed and fasted rabbits. In considering the data of Table II it must be remembered that all values are relative and that in reality all component fatty acids decrease on starvation. The meaning of an increase in percentage composition of any particular component is only that it is not disappearing as rapidly as are the other components. From this table it is seen that the unsaturated acids represent 62% of the marrow fat, with linoleic

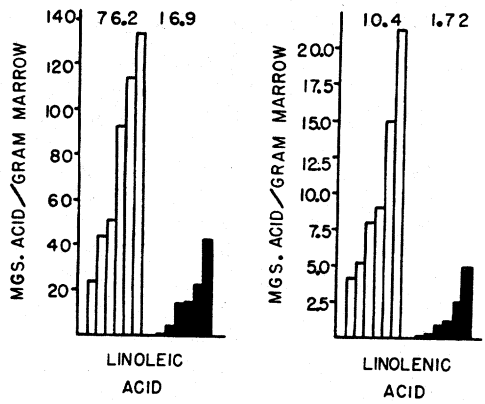

FIG. 1. Linoleic and linolenic content of marrows from individual rabbits. Unshaded vertical columns represent fed rabbits; blackened columns, unfed animals.

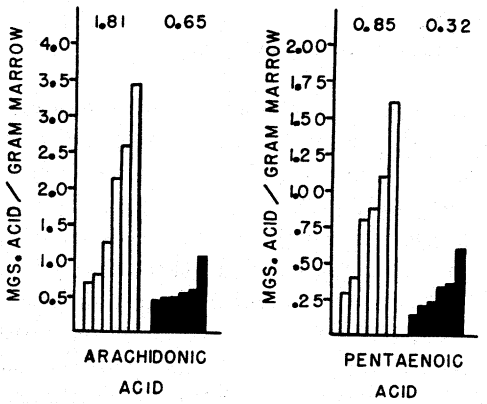

FIG. 2. Arachidonic and pentaenoic acids content of marrows from individual rabbits.

acid alone comprising as great a percentage as the sum of the saturated acids. The data suggest that the principal reason for a decrease in the iodine number of marrow fat during fasting is the preferential disappearance of linoleic acid.

Oxygen consumption rates of marrow cells from fed and starved animals are shown in Table III. Agreement of rates from the same animal averaged 2%, but great discrepancy occurred when results from different animals were compared, particularly in regard to the fed rabbits. Marrows from unfed rabbits continued to show sizable oxygen consump-

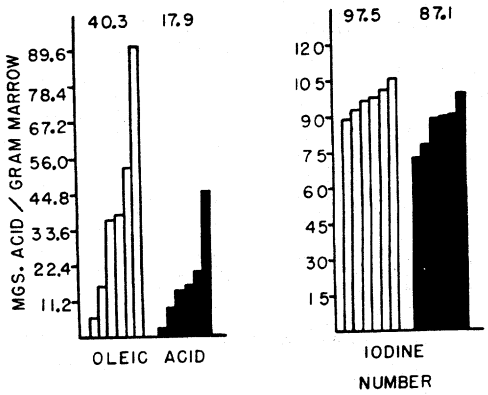

FIG. 3. Left figure shows content of oleic acid in marrows of individual rabbits. Right figure shows the iodine number of fat extracted from the marrows of experimental animals.

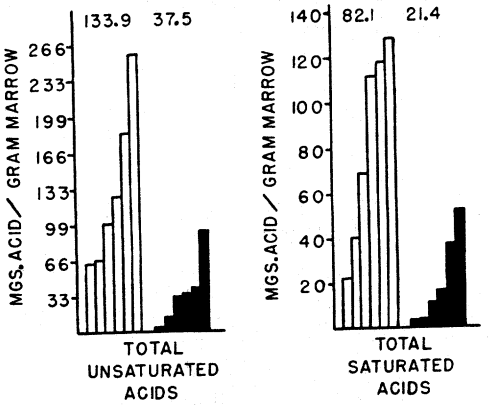

FIG. 4. Total saturated and total unsaturated fatty acids in the marrows of individual rabbits.

tion, even though they were almost devoid of fat. For example, in one fasted animal in which lipide material constituted less than 1% of the total marrow weight, the rate of oxygen consumption was still 0.24 cu. mm./mg./hr. during the 4-hr. period over which it was studied. When calculations were made relating oxygen utilization rates to total fat, no correlation was found, the computed values varying between 0.18 and 28.3 cu. mm./mg. total fat/hr. Table III shows that rabbit marrows containing large concentrations of total polyunsaturated acids do not

TABLE II

Percentage Distribution of Fatty Acids in Marrow Fat of Fed and Fasted Rabbits

Fatty acid	Fed (average)	Fasted (average)
Linoleic	33.0	25.2
Linolenic	4.5	2.3
Arachidonic	0.8	1.9
Pentaenoic	0.3	0.8
Oleic	23.6	31.7
Total unsaturated	62.3	61.8
Total saturated	33.4	34.0

TABLE III

Summary of Data on Total Polyethenoid Acid Content, Oxygen Consumption and Iron Content in Marrows of Individual Rabbits

Exptl. No.	Total polyunsaturated fatty acids	Oxygen consumption	Iron
	mg./g. raw marrow	*cu. mm./mg. raw marrow/hr.*	*μg./g. raw marrow*
Fed rabbits			
1	29	—	88
2	51	0.34	43
3	61	0.34	90
4	102	0.10	—
5	131	0.11	—
6	165	0.07	—
Fasted rabbits			
7	2	0.24	106
8	7	0.34	—
9	17	0.21	146
10	18	0.34	—
11	26	0.36	—
12	49	0.34	155

give large oxygen consumption rates; similarly, marrows with the lowest concentration of polyethenoid substances are not associated with minimal rates of oxygen consumption. Further detailed examination of the data reveals that no constant relation exists between oxygen consumption rate and the quantity of any unsaturated or saturated fatty acid component of the marrow.

In the earlier experiments in this series it was observed that the marrows from fasted rabbits were frequently excessively blood filled. Analyses showed that in three normally feeding rabbits the iron content (see Table III) averaged 73.6 μg./mg. raw marrow. Similar analyses of

TABLE IV

Effect of 20-hour Incubation on the Composition of Marrow Fat (Four Rabbits)

	Not incubated	Incubated
Linoleic acid, %	28.15	28.28
Linolenic acid, %	3.34	3.43
Arachidonic acid, %	0.92	0.92
Pentaenoic acid, %	0.29	0.31
Oleic acid, %	25.6	24.4
Total unsaturated acid, %	58.3	57.3
Total saturated acid, %	37.4	38.4
Iodine number	87.3	86.8
Peroxide number	Trace	3
Saponification equivalent (as glyceride)	292.6	295.2
Free fatty acid (as per cent oleic acid)	0.56	0.60

the marrows of three unfed rabbits showed an average iron content of 135.6 μg./g. marrow. It appears that the iron content and therefore the hemoglobin content may be elevated as much as 84% during prolonged fasting. The accumulation of hemoglobin in marrow under conditions of undernutrition (7) may be of interest in view of the known catalytic action of hemoglobin on polyunsaturated acids (8, 9).

In three experiments marrow was incubated aerobically at 38° for 20 hr. during which time the oxygen consumption rate remained essentially constant. Immediately afterward the fat was quantitatively recovered from the contents of the Warburg vessels and was analyzed spectrophotometrically. Comparison of the analyses from incubated and nonincubated marrow samples showed no significant differences in fatty acid composition. Data from one typical experiment are given in Table IV. The marrows from four normal rabbits were combined to give a sufficient amount of fat for determinations of saponification equivalent, peroxide number, and free fatty acid.

Discussion

The composition of the saturated fat of normal bone marrow has been described (10), but quantitative estimations of the unsaturated components have not been made although several investigators (11, 12) have suggested from iodine-number determinations that marrow contains polyunsaturated acids. Kies and Webster (13) identified linoleic acid in rabbit marrow. The spectrophotometric method used in the present investigation permits quick, accurate analyses on fat samples containing as little as 5 mg. Duplicate analyses for each unsaturated acid uni-

formly agreed within 0.5 % when expressed as per cent by weight of the total fat. Analyses of the same marrow fat performed on different days gave results that agreed within 1 %. The very precision of the method emphasizes the variations in the marrows from both fed and fasted rabbits.

One unique characteristic of rabbit marrow is its capacity to utilize oxygen *in vitro* for as long as 24 hr. This large and prolonged consumption is not attributable to carbohydrate (14) nor is it accompanied by any unusual protein breakdown as determined by semimicro Kjeldahl analyses. The present experiments show that the rate of oxygen utilization is not proportional to the total quantity of marrow fat nor to the concentration of any single fatty acid component. However, in all the marrows studied, including those that were most depleted of fat, there was still sufficient lipide material to support the oxygen consumption experimentally obtained. For example, one marrow sample from a profoundly starved rabbit consumed 265 cu. mm. oxygen in 4 hr. in a Warburg flask. The sample contained 2.35 mg. lipide, an amount which if burned completely to carbon dioxide and water would require approximately 4750 cu. mm. oxygen. Yet this was the leanest marrow we obtained. Differential manometry supports the concept of complete combustion inasmuch as respiratory quotients approximate 0.85 over a 4-hr. experimental period. If one assumes incomplete combustion with only one oxygen molecule combining with each fat molecule, the amount of of oxygen required would be approximately 200 cu. mm.

From such considerations it appears theoretically possible that a large fraction of the oxygen consumed *in vitro* might be associated with the combustion of fatty substances. However, the data of Table IV strongly suggest that neither the unsaturated nor saturated fatty acids deteriorate under our experimental conditions. The evidence pertaining to the unsaturated acids is particularly noteworthy because polyethenoid fatty acids are unstable in oxygen especially in the presence of heme or heme-containing compounds. Not only does the chain reaction of autoxidation not begin in these incubated marrows, but even peroxidation of the unsaturated fat does not get under way, apparently because of the presence of natural inhibitors. Determinations of preformed conjugation in samples of incubated and nonincubated marrow show negligible values, thereby supporting the other data of Table IV. We interpret our results to mean that the polyunsaturated acids of marrow are not oxidized *in situ* and therefore disappear from marrow during fasting because they are carried away by some transport system. The constancy of saponifica-

tion equivalents suggests that marrow fatty acids, on prolonged incubation, do not change into other fatty acids of lower molecular weight, at least on any large scale. Likewise, from the amounts and constancy of the free fatty acids in our samples, it appears that even during long-continued aerobic incubation no mechanisms split the marrow glycerides into their basic components. Our interpretation of these facts is that the saturated fats are also probably not degraded in the marrow for the production of local energy.

Newlin and McCay (12) reported that marrow fat reflected the quality of a rabbit's diet, and they regarded marrow as a reservoir permitting ready deposition and withdrawal of fatty substances. The present experiments do not give specific information about the metabolic fate of any of the fatty acids in normal marrow, but they point out clearly the large quantity of polyunsaturated acids in normal marrow and emphasize the rapidity with which each component disappears during starvation. In the light of evidence presented here, it seems likely that polyunsaturated acids of bone marrow play a more active role in metabolism than they have hitherto been assigned.

Summary

1. Spectrophotometric analyses of the marrow fat of six fed and six fasted rabbits have been compared with their respective *in vitro* oxygen consumptions.

2. Unsaturated fatty acids constitute 62% by weight of the total marrow fat. The quantity of linoleic acid in marrow from fed rabbits is as great as that of all combined saturated fatty acids.

3. The absolute quantities of all components of marrow fat diminish during starvation. The per cent of linoleic and linolenic acids decreases during fasting, whereas that of arachidonic, pentaenoic, and oleic acids increases. In terms of per cent, total saturated fatty acid in marrow fat is essentially unaffected by starvation.

4. Rate of oxygen consumption *in vitro* is not related to the concentration of any fatty acid component of marrow fat.

5. It is concluded from *in vitro* evidence that polyunsaturated fatty acids of marrow are not oxidized *in situ* but are transported elsewhere for utilization or discard.

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